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INFORMATION BULLETIN

New Leitz Micro Manipulator

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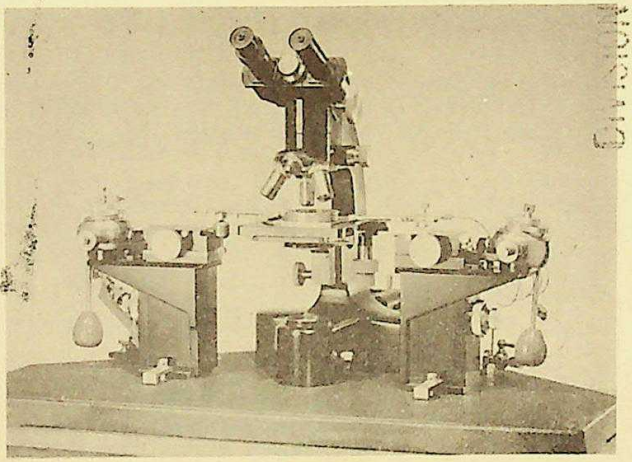

Ever since the invention of the microscope, research workers have attempted to carry out dissections within the microscopic field of view. The observation of living cells by means of phase contrast or under flourescence has brought science closer to the solution of questions in the field of biology and medicine. Micro-surgery, carried out by means of the new Leitz Micro Manipulator, is another means of getting closer to the solution of these same questions.

Even under the most favorable conditions, micro-surgery takes place in a space of very few millimeters, in most cases, in fact, within fractions of millimeters. The transmission of the normal movement of the human hand in such small dimensions is possible only with aids which control these movements accurately and which eliminate all vibrations. With the development of this new micro-manipulator, Leitz has brought on the market an instrument which will meet even the most exacting requirements. It should be remembered that the field of view of an objective 3.5x and an eyepiece 6x is only 5.6mm in diameter, while the field of view of an oil immersion objective 100x and a Periplan eyepiece 10x is but 0.136mm in diameter.

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The manufacture of micro-manipulator equipment goes back to the year 1844. In 1921 Chambers designed an instrument which produced very fine movements without lost motion. The field of application for micro-manipulators can be summarized as follows:

1. In the field of Biology and Medicine: Micro surgery, the production of single cultures, artificial insemination, the isolation of organisms or parts of organisms and their transplanting, the isolation of single sperms or bacteria, and the injection of media.

2. In Technology: The examination of: constituents of materials, the elasticity of fibers, the measurements of viscosity, the manufacture of extremely fine scales, the assembly of very fine mechanical devices, and others.

3. In Metal and Ore Research: For the orientation of extremely fine particles, for instance, to orient metal filings or minerals for the purpose of taking electron optical photographs, and many others.

The research worker desires to use such an instrument under all normal microscope magnifications. The micro instruments (assistants) must be guided so accurately and precisely that the necessary operations can be carried out on any part of an object seen in the field of view and which may measure only fractions of microns. It may be desirable, for instance, to use micro pipettes, micro knives, micro needles, or micro tweezers to remove the nucleus from a single cell or from parts thereof. It is also desired to inject experimentally the smallest quantity into the nucleus of cells in order to study the effect of these injections on the nuclei or the plasma. This method is of great importance for the investigation of the origin of infections. The progress which has been made in the physiology of genetics would be unthinkable without a method for ex- and implantation.

The micro-manipulator, therefore, has been developed in response to a real need. In designing the new Leitz Micro Manipulator, the thought has prevailed that the practical research worker will prefer an instrument which will conduct the movement of the hands by a single lever action and that meets the following requirements.

A. It should be possible to reach any desired point within the field of view under the highest magnifications of 1,000x without changing the position of the hand. To combine all three space co-ordinates into one lever action proves to be unsatisfactory because to adjust the height under the highest magnifications with the same lever can hardly be done without changing the adjustment from side to side.

B. The movements of the tools (assistants) should take place symmetrically and in the same form and the movement should be reduceable to the desired field of operations. This latter requirement is one of the most important ones, because it eliminates the possibility that the "assistants" will inadvertantly be guided out of the field of operations while working with the manipulator. The hand should be enabled to always move as it would without a manipulator.

C. The micro-manipulator should react instantly upon any movement of the hand and it should not be subject to variences which are common in pneumatically operated designs. The fine adjustments should operate completely independently of the coarse adjustments.

The following is to be said about the manipulator accessories (assistants):

A. The "assistants" ought to be easily interchangeable and it should be possible to set them in any direction either against one another or against the object. In other words, it must be possible to move an "assistant" at every possible angle within space towards the object.

B. In a double manipulator holder there must be two "assistants" which act like tweezers and allow co-ordinated movements vertically as well as horizontally. It is necessary that these movements are independent of the actual manipulator and that they can be carried out with the same degree of precision.

C. Furthermore, there are requirements for the mechanical manufacture of "assistants". In the simplest cases, it is sufficient to have a needle puller for the mechanical manufacture of pipettes and a micro burner for the preparation of other instruments by hand. A so-called micro forge is desirable for the most precise instruments, and these are made under observation through a stereo binocular microscope.

The Leitz Micro Manipulator is supplied with a base plate which in its center receives the microscope stand and one each micro-manipulator unit is placed to the right and to the left of the microscope.

We are submitting hereunder specifications for the basic micro-manipulator equipment which is recommended for general research work.

Specifications:

One Micro Manipulator with the accessories for right hand operation.
One Micro Manipulator with the accessories for left hand operation.
One base plate 50 x 40 x 2cm, accommodating the microscope and the manipulators.
One needle holder for one needle.
One needle holder for two needles.
One injection apparatus with glass syringe on socket for 2 cubic cm., metal tubing and three-way valve, rubber tubing and mouth pipette.

Leitz Micro Manipulator basic equipment, as listed above.... $ 1,660.00

Accessories available upon request for brightfield observation:

Brightfield condenser with long free-working distance of 15mm with a numerical aperture of 0.75 - complete in case..... $ 24.00

Moist chamber made of metal with two openings including metal ring for closing one of the two openings and including two round bottom cover glasses, - in case..... 28.00

One dozen round cover glass plates 54mm in diameter, 0.17mm thick..... 5.00

Accessories for brightfield observation, complete..... $ 57.00

Accessories available upon request for darkfield observation:

Darkfield condenser with numerical aperture 0.75 - 0.80 with two openings for the insertion of micro instruments, 10mm high, one special round stage plate with an opening 50mm in diameter. One special metal chamber, with case, accommodating the complete equipment..... 180.00

NOTE: When ordering a darkfield condenser for an existing microscope, it is essential to state the type of instrument and the serial number so that the proper darkfield condenser can be supplied.

Further Accessories:

Remote control unit (flexible shaft) for operating the microscope fine adjustment from the base plate of the micro-manipulator..... 19.00

Although standard Leitz monocular and binocular microscopes can be used with the Micro Manipulator, we recommend the following special manipulator microscope:

Leitz microscope model Laborlux II 46/76, with built-in source of light 6 Volts, 5 Amperes, with coarse adjustment by rack and pinion motion, fine adjustment by means of dual micrometer screw, both located in the upper arm of the microscope stand.

Tube changing device, square mechanical stage #46 with graduations, two diaphragm swing-out condenser after Berek, N.A. 0.90, with centering device, with inclined binocular tube S.

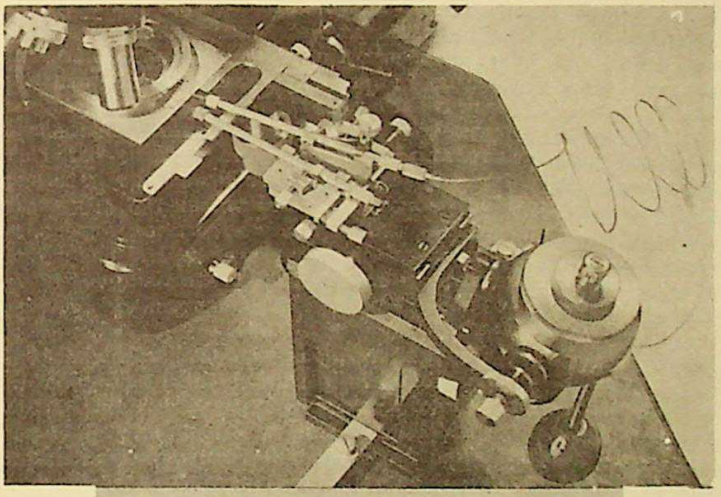
Microscope, complete in cabinet.....LOALK 647.00

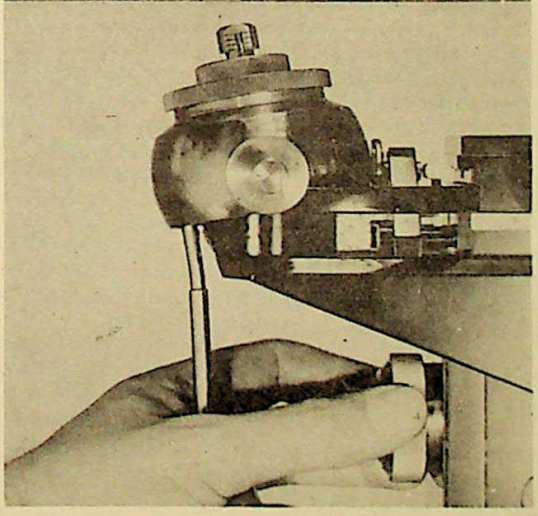
Remote control unit (flexible shaft) to operate the fine adjustment of the microscope from the manipulator base plate. 19.00

Optical Equipment consisting of:

Revolving nosepiece for four objectives	QUATO	$ 20.00
Achromatic objective 3.5/0.10	OANEE	16.50
Achromatic objective 10/0.25	ACORA	20.00
Achromatic objective 45/0.65	ACMAT	32.00
Achromatic oil immersion objective 100/1.32 ..	OILIM	57.00
Paired Huyghens eyepieces 6x	HYZWA	12.00
Paired Periplan eyepieces 8x	GIROT	24.00
Paired Periplan eyepieces 12x	GIZOV	26.00
Regulating transformer for the built-in light source	REDYX	45.00
Total Optical Equipment		$ 252.50

Directions for Use of the Micro-Manipulator





In most cases, the two micro-manipulator units are placed on dove-tail sliders to the right and left of the microscope proper, and as soon as both manipulators are adjusted to the same height, the points of the two "assistants" will show up in the middle of the field of view. All parts, namely the microscope, the micro manipulators, the micro injection apparatus, etc., are fastened firmly to the base plate.

The "assistants" are placed in holders which are supported by ball sockets. The holders on the manipulators are rotatable, the desired direction can already be established when the holders are fastened. The vertical coarse adjustment of the manipulators corresponds to the coarse adjustment of the microscope. The horizontal coarse adjustment can be adjusted in such a way that it is possible to remove an "assistant" and to bring it back into the field of view by simply operating the coarse movement. The fine adjustment of the "assistants" in the vertical direction corresponds to the fine adjustment of the microscope. In the horizontal direction, this fine movement is carried out by means of a ball segment which is operated through a guiding rod with a ball at its end. Each movement of the hand causes an eccentric movement of the segment and is thus transmitted to the "assistants." By raising and lowering the eccentric ball segment, the ratio of transmission may be varied continuously from 16:1 to 800:1. The hand which operates the manipulator carries out essentially the same movements under all magnifications, both with respect to direction and relative size. The extent of the movements always remains within a pre-selected field of operation. The index marks allow to relocate the chosen ratio.

Single and double instrument holders are provided for the "assistants"; they are built on a new principle which permits easy and quick interchange of the shafts which hold the "assistants". The free forward end of the shafts is conical; it has an opening of approximately 3mm and the "assistant" is introduced into this opening (either a hollow or a solid needle of 1mm Ø) and held there securely by means of a valve.

The culture chamber consists of a rectangular plate, which at the same time closes it at the bottom and serves as its support. The chamber is closed by means of a large cover glass. The height of the chamber is always 7mm measured from the stage. It makes no differance whether one chooses the chamber itself to be 6mm and the slide 1mm, or the chamber 1mm and the slide 6mm, or whether still other measurements are chosen. The condenser which is supplied has a long focal length, so that a good brightfield is achieved with standard objectives. The "Heine" phase contrast condenser can be interchanged against the brightfield condenser and allows observations in brightfield, phase contrast, and darkfield with all Pv dry lenses, and in brightfield and darkfield with all standard objectives up to a numerical aperture of 0.70.

The oil immersion Pv 70/1.15 permits observation under brightfield and phase contrast only.

The setting up of the micro-manipulator is different depending on whether one uses a microscope with a fixed tube or one which can be raised and lowered. The table which follows shows the difference.

MICRO MANIPULATOR SETTING

Microscope with fixed tube	Microscope with tube which raises and lowers
1) Focus the object; stage movement; coarse and fine adjustment (microscope).	1) Movement of the "assistants" to the field of view; operation by means of the micro manipulator.
2) Movement of the object by means of the mechanical stage.	2) Movement of the "assistants" by means of the coarse adjustment.
3) Adjust the height and the movement of the "assistants" to field of view by means of the micro manipulator movements.	3) Focus the desired object detail by means of the tube using microscope coarse and fine adjustment.
4) Withdraw the "assistants" by means of the coarse adjustment.	4) Height of the "assistants" ought to be adjusted; bring them into the field of view by means of the micro manipulator movements.
5) Bring the object back to position 1 by means of the mechanical stage. Since in this case the last movements were carried out by means of the coarse adjustment, it will become necessary to use the fine adjustment later on. (See below A-C)	

If new focusing during operation becomes necessary, the following movements are suggested:

Microscope with fixed tube	Microscope with tube which raises and lowers
A) Remove the "assistants" from the object by means of the micro adjustment.	A) Readjust the microscope tube so that the desired object detail is in focus by means of the micro adjustment.
B) Bring the object in focus by means of the micrometer screw.	B) Refocus the "assistants" by means of the micrometer screw.
C) Readjust the "assistant" by means of the micrometer screw. The movement B must be smaller than the distance between "assistants" and slide.* In most cases, the adjustment has to be carried out one after the other in succession.	

*(or "assistants" and cover glass)

Base Plate: The microscope is always placed in the middle of the base plate and fixed in position. Generally, the two manipulators are placed into their dovetail guides to the right and to the left; in other words, one on each side of the microscope so that the "assistants" can by means of the coarse adjustment be removed from the field of operation. This means that the base plate upon which the "assistants" are fixed is so set that the 3rd upper index mark is near the first lower index mark. Then the micro manipulator stand is securely fastened. The injection device can be fastened either to the right or to the left, or one can even place two on both sides.

Injection Device: The injection device consists of a glass injection syringe, a holding socket, and a screw guide, a 3-way stop cock, capillary tubes made of flexible synthetic material and the pipette holder. The length of the tubing may be chosen as desired. The tubing and syringe are filled with distilled water, making sure that no air bubbles remain. The water serves only one purpose, namely to promulgate the pressure that is put on the syringe to the micro pipette. Before filling the micro pipette with the injection fluid, it is recommended that a neutral medium be suctioned between the distilled water and the culture solution. For simple operations, a mouth pipette is all that is generally needed. The latter consists of a glass mouth piece, a synthetic tubing and a pipette holder for the micro glass pipette.

Culture Samples: A so-called wet chamber with two openings for the insertion of micro instruments may be chosen or the so-called oil chamber. A disadvantage of the wet chamber is that due to the form of the hanging drop, phase contrast and darkfield are possible only in the middle of the field of operations. The chamber itself consists of rust-free steel with glass between it and the cover glass. It has to be kept absolutely sterile. The sides can be laid out with wetted paper, and the opening may thus be closed in order to safeguard the culture as much as possible from evaporation. The oil chamber is made of glass. There are two square glass rods cemented onto a glass plate. It is filled in the following manner. The cover glass is wetted in the middle with the culture solution, and then a large drop of absolutely neutral and very fine parafin oil is placed over it. The pipette now serves to inject the culture solution underneath the oil. The cover glass is turned upside down and is placed on top of the chamber. The oil drop should be of such size that the chamber is now filled with oil both from below and above. A pipette serves to add culture solution so that the entire wetted area in the culture is occupied. The drop with the culture solution will now expand and because of the oil in the chamber, it will remain flattened. The culture can be continuously renewed. Furthermore, it is possible to insert into the culture oxygen or glucose by means of capillaries. This method is much easier with modern microscopical possibilities because in this way the lens effect of the hanging drop is greatly eliminated because the culture usually hangs on to the cover glass. As far as the inside depth of the chamber is concerned, one would not go beyond 5mm because otherwise the movement of the fine needles would become very much hampered by the solution. We supply chambers with 3mm or one with 5mm lumen. "Assistants" have to be made up for intended purposes by the ultimate users.

Assistants: Small glass capillaries of 1mm in diameter are pulled by means of a needle puller or by hand to approximately 1/10 of its thickness. It is important that the capillaries narrow quickly. Then if the capillaries are to be pulled still further, they are then weighted and brought near a very fine burner in order to be pulled still finer. Depending upon the size of the weights, the lumen capacity of the pipettes the narrowing can be chosen as desired. If needles or other instruments have to be prepared, a solid glass needle is brought in contact with a molten glass drop, and the end is pulled as desired. After a certain amount of experience, any form as well as any thickness can be achieved. A micro forge, of course, is necessary for this purpose, and with it a stereoscopic microscope, a rotating stage with interchangeable glass micro burners, and a holding device for the glass needles which are to be pulled.

Condensers: With the micro manipulator, we generally supply the standard two-diaphragm condenser with a cap for counting chambers. This affords a long working distance and an illuminating aperture of 0.75. For observation in brightfield, darkfield and phase contrast, we recommend the phase contrast condenser after Heine. It is possible to observe in the oil chamber under darkfield up to an illuminating aperture of 0.70.

Objectives & Eyepieces: It is recommended basically that objectives with long working distance are used. It is always preferable to choose the objective with the lower magnification and the larger free working distance than vice versa. We recommend the following optical outfits:

* BF, DF:	3.5/0.10 (F.W.D. 23mm)	BF, DF: Pc 10/0.10 (F.W.D. 5.8mm)
BF, DF:	6/0.10 (F.W.D. 17mm)	BF, DF: Pc 20/0.25 (F.W.D. 2.0mm)
BF, DF:	10/0.25 (F.W.D. 5.7mm)	BF, DF: Pc 40/0.70 (F.W.D. 0.38mm)
BF, DF:	45/0.65 (F.W.D. 0.6mm)	BF, DF: Pc 70/1.15 (F.W.D. 0.2mm)

*Note: BF - Brightfield
DF - Darkfield
Pc - Phase Contrast
FWD - Free Working Distance

Microscopes: We recommend for micro manipulation all Leitz microscope stands including the Panphot. The manipulation is, of course, slightly more difficult with microscopes which have stages that can be focused up and down. Also, the Greenough microscope is sometimes used for magnifications under 200x.

Generally, we supply the micro manipulators completely together with our Labolux II model microscope.

Cover Glasses, Glass Tubing and Glass Rods: The size of the cover glasses depends on the oil or wet chamber. It is important that in working either objectives of 20x and more, the thickness of the cover glass remains 0.17 mm. With glass tubes and glass rods, always observe whether their outside diameter is exactly 1mm.

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